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This is a News and Views comment on the following paper:
Saunders, J. C. et al. an in vivo platform for identifying inhibitors of protein aggregation. Nature Chemical Biology 1–11 (2015). doi:10.1038/nchembio.1988

A rescue by chaperones

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Protein aggregation is associated to more than 50 human pathologies including prevalent diseases such as Alzheimer and Parkinson disease. A phenotypic screen in *Escherichia coli* associating antibiotic resistance to the inhibition of protein aggregation now allows screening for chemical inhibitors of protein aggregation in a simple, fast and inexpensive manner.

Protein misfolding and aggregation have been associated to a growing list of pathologies including neurodegenerative diseases, systemic and organ-specific amyloidoses[1] but also metabolic diseases and cancer[2]. Protein misfolding and aggregation result in disease through protein loss-of-function but additionally, aggregated proteins often also acquire toxic properties through mechanisms that still remain unclear. What is known however is that aggregation results from the self-assembly by β-strand interactions of short aggregation-prone amino acid segments within a protein chain[3]. When a protein is in its native conformation these aggregation sensitive sequences are generally buried in the hydrophobic core where they are protected from aggregation by numerous native interactions. Familial aggregation diseases such as lysozyme amyloidosis are caused by mutations that destabilize the native conformation of the protein thereby increasing the fraction of solvent-exposure of the aggregation-prone protein segments. However, for many aggregation-related pathologies, including Alzheimer's and Parkinson's, the sporadic form of the disease is the most prevalent by far. Here the wild type protein is affected by aggregation as a result of age-related decline in the ability to efficiently remove misfolded proteins, for instance during processes such as protein synthesis and translocation[4].

One of the main strategies explored to treat aggregation-related diseases consists of identifying chemical chaperones, i.e. compounds that inhibit protein aggregation by direct interaction with the affected protein[5]. Chemical chaperones can be envisaged to work by several means. First, chemical chaperones could inhibit protein misfolding by (kinetic) stabilisation of the native structure. This strategy has lead to the development of Tafamidis[6] that inhibits mutant transthyrethin amyloidosis, but is also being explored for several lysosomal storage disorders[7] such as Gaucher disease. Second, chemical

chaperones could also inhibit or modify the self-assembly of aggregation-prone sequences thereby lowering aggregate toxicity or facilitating their degradation by the cell.

As promising as chemical chaperones appear, their development is fraught with practical and technical obstacles. As a result only one chemical chaperone has made it to the market so far. One major barrier is the disordered nature of many disease-associated aggregating protein impeding strategies aiming at stabilizing native structures. Further, *in vitro* assays require relatively large amounts of purified protein, which can be limiting when working with aggregating proteins. Moreover, these assays typically monitor aggregation using conformational dyes such as ThT that can potentially interfere with compound binding and efficacy.. Finally, it is often difficult to produce homogenous starting samples consisting only of non-aggregated protein, affecting experimental reproducibility. Perhaps counterintuitively, *in vivo* assays in yeast or mamalian cell culture also have important limitations when it comes to protein aggregation. Many aggregation diseases involve extracellular aggregation. Assays requiring cellular uptake will therefore limit the available chemical space, while the reducing environment of the cytoplasm is not ideally suited to screen for aggregation in an oxidative milieu.

In this issue Saunders et al[8] have pushed forward an in vivo assay in E.coli they previously developed to screen for mutations that optimise protein stability in E. coli[9] and cleverly adapted it to allow screening for the inhibition of amyloid aggregation. Originally, the assay consists of a tripartite β-lactamase fusion construct linking the activity of split β -lactamase and thus ampicillin resistance to the structural stability of the target protein (Figure 1). However, as mentioned above many aggregating proteins involved in human disease are intrinsically disordered and their aggregation-prone sequences cannot be protected from aggregation by stabilizing the native state. Expressing the Islet Amyloid Polypeptide and the Alzheimer β-amyloid peptide the authors demonstrate that the same tripartite β -lactamase fusion can be used to link the amyloid assembly of aggregation prone sequence peptides to antibiotic resitance. They further validate the utility of this phenotypic assay for screening compounds that modify the aggregation of IAPP. An additional advantage of the assay resides in the periplasmic localisation of the reporter system which provides both compound accessibility and an oxidative environment.

A potential danger from a reporter screen on antibiotic resistance is the possibility that hit compounds do not act directly on the aggregating protein of interest, but instead alter proteostasis of the periplasm. The authors addressed this by coupling their phenotypic screen to electrospray ionisation-mass spectrometry linked to ion mobility spectrometry (ESI-IMS-MS)[10] analysis of hit compounds, a method that provides a snapshot of the complex soup of species that accumulates during the aggregation process. This not only allows to discern compounds that directly bind to the target protein, but also provides information on exactly what aggregating species is bound and how this affects the composition of the reaction mixture. The combination of both these techniques creates an exciting opportunity to search for novel molecules

that can modulate protein aggregation, but also highlights the challenge of creating chemicals that combine the seemingly contradictory properties of specifically interacting with hydrophobic surfaces involved in aggregation while at the same time maintaining a sufficient level of solubility.

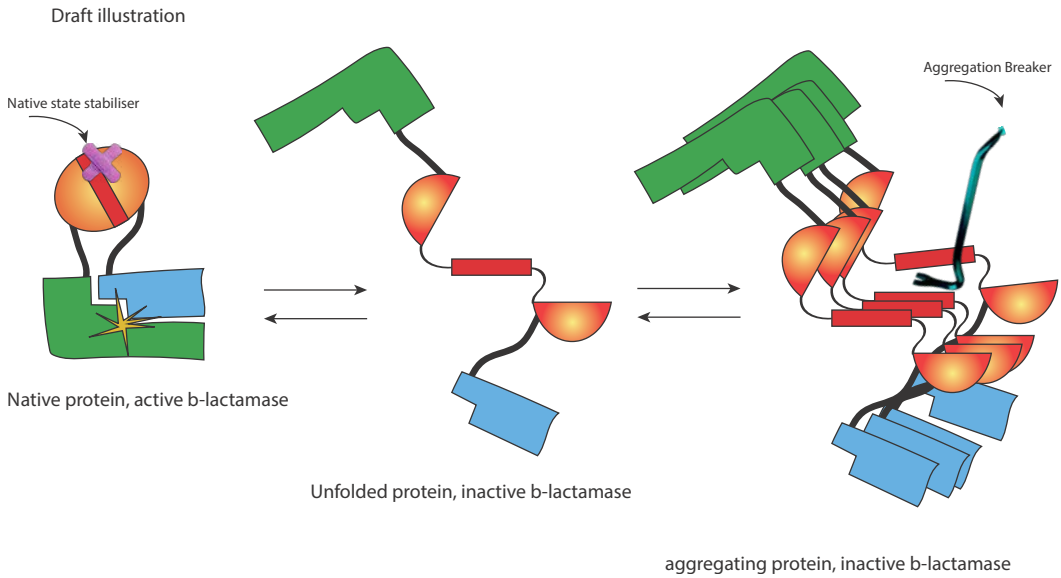

Figure 1: Tripartite β-lactamase screening assay against aggregation. β-lactamase function, and thus ampicillin resistance, is restored when aggregation of the target protein is inhibited. This can occur either through stabilization of the native structure (left) or through inhibition of the process of amyloid self-assembly (right).

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